REMARKS/ARGUMENTS

In the specification, the title has been amended to more closely reflect the subject matter of the present invention. The new paragraph added at page 1 sets forth the priority claim to provisional application 60/199,545, filed April 25, 2000, which priority claim was timely made in the Oath and Declaration filed August 1, 2001 in the present application. A copy of the corrected filing receipt setting forth the priority claim is enclosed herewith as Exhibit A.

Rejection under 35 USC § 112, First Paragraph

Claims 1 - 4 and 6 - 12 were rejected under 35 USC § 112, first paragraph, as the specification allegedly does not enable one skilled in the art to use the invention. According to the Examiner, it has been art-recognized that immunotherapy to cancer has been limited. The Examiner asserts that, with respect to "treating a precancerous subject" there is insufficient guidance to enable the skilled artisan to choose such "precancerous" subjects. The Examiner further states that with respect to the use of CD30L, the instant specification appears to be lacking in the teachings of how to make and use CD30L to treat tumors, in that it does not appear to distinguish treating large cell anaplastic lymphoma with unlabeled CD30L versus treating CD30 expressing tumors with CD30L conjugates. Applicants respectfully disagree.

At the outset, Applicants respectfully assert that the animal model utilized in the present application is regarded by those of skill in the art as reasonably predictive of results that could be obtained in humans, and reserve the right to present evidence to that effect if the Examiner rejects the application on those grounds.

With respect to the Examiner's assertion that there is insufficient guidance to enable one of ordinary skill in the art to choose precancerous subjects, Applicants submit herewith as Exhibit B a printout of a search of a public database for English language reviews on human subjects, published before February 2000, with 'precancerous' in the Title. As these review articles show, those of ordinary skill in the art were well-aware of precancerous states at the time the present application was filed, and thus were able to select subjects afflicted with precancerous conditions. Moreover, Applicants disclosed Barrett's esophagus as an exemplary precancerous condition for which photodynamic therapy (see page 12, at lines 20 – 21). The instant application is thus enabled for

precancerous subjects, and Applicants respectfully request that this aspect of the rejection be withdrawn.

Applicants submit that those of ordinary skill in the art know how to make and use CD30L in the presently claimed invention. In the specification, at page 2, lines 22 and 23, Applicants state that CD30L is a suitable agent for therapeutic use in conjunction with the invention. Its suitability is based on its activity as a stimulus for proliferation of activated T cells, as disclosed in the Goodwin et al. patent, the paragraph spanning columns 2 and 3, and in Example 8 and 13 (which begin at columns 30 and 34, respectively). Thus, the use of CD30L as a T cell stimulus was well-known n the art at the time the present application was filed. Accordingly, Applicants request that this aspect of the rejection be withdrawn.

Rejection under 35 USC § 103(a)

Claims 1, 2, 4 and 6 – 12 were rejected under 35 USC § 103(a), as allegedly being unpatentable over Curry et al., and/or Hunt et al., in view of Armitage et al. (US Patent 5,674,492) and/or Lynch et al., and further in view of Armitage et al. (US Patent 6,410,711). According to the Examiner, Curry et al. teach the combination of photodynamic therapy and immunoadjuvants, and Hunt et al. teach the combination of photodynamic therapy and apoptosis-inducing agents. The Examiner states that Curry et al. and Hunt et al. differ from the claimed method by not disclosing the use of CD40L or CD30L as an adjuvant of apoptosis-inducing agent. Armitage et al. ('492), Lynch et al. and Armitage et al. ('711) are cited to cure these deficiencies. Applicants respectfully disagree that the combination of references set forth by the Examiner teaches or even suggests their invention.

Applicants assert that they are entitled to a priority date of April 25, 2000, the filing date for a provisional application from which the present application claims benefit. The Curry et al. reference is a published US Patent Application that is a continuation-in-part (filed Jan. 9, 2001) of a non-provisional application (filed Apr. 21, 2000) of provisional application 60/130,519 (filed April 23, 1999). A copy of the provisional application is enclosed herewith as Exhibit C.

While a listing of adjuvants contemplated by Curry et al. is given in Appendix A of the provisional application, absent from this list, and from the provisional application itself, is any discussion of biological response modifiers such as cytokines. In contrast, the published Curry et al. application specifically discusses cytokines, for example, at

paragraph [0051]. Applicants submit that this material was added to the continuation-in-part filed Jan. 9, 2001, which date is after Applicant's claimed priority date.

Moreover, Curry et al. neither teaches nor suggests the combination photodynamic therapy with the biological response modifiers (i.e., CD40 binding proteins) set forth in the presently claimed invention. At best, Curry et al. might be regarded as an invitation for one of skill in the art to contemplate whether other cytokines might be useful in combination with photodynamic therapy but it is completely silent on what those cytokines might be. Furthermore, even this vague hint was not taught by Curry et al. until after Applicants had already filed an application disclosing the present invention.

Hunt et al. discloses and claims the use of photodynamic therapy in combination with apoptosis-inducing agents. They note that treatment of target cells with apoptosis-inducing agents either before, after or during photodynamic therapy results in enhanced target cell destruction. However, Hunt et al. neither teach nor disclose the use of biological response modifiers, in particular CD40 binding proteins, to enhance an immune response to the target cells. Rather, the crux of Hunt et al.'s invention lies in the increased destruction of target cells by their claimed combination. There is neither a teaching nor a suggestion that one of skill in the art should use a CD40 binding protein (or any other biological response modifier) to enhance an immune response in conjunction with photodynamic therapy.

Neither of the Armitage patents, nor the Lynch et al. publication cures these deficiencies. Not one of these publications discloses or even suggests the use of photodynamic therapy, nor do they disclose the use of CD40 binding proteins as apoptosis-inducing agents. Applicants respectfully submit that there is no motivation in the art to make the combination of references set forth by the Examiner, nor an expectation of success in so doing. Accordingly, Applicants request that the rejection be withdrawn.

Claim 3 was rejected under 35 USC § 103(a), as allegedly being unpatentable over Curry et al., and/or Hunt et al., in view of Armitage et al. (US Patent 5,674,492) and/or Lynch et al., and further in view of Armitage et al. (US Patent 6,410,711) as applied previously and further in view of Goodwin et al. The teachings of Curry et al., Hunt et al., Armitage et al., Lynch et al. and Armtage et al. were as set forth in the previous rejection. According to the Examiner, Goodwin et al. teach that CD30L conjugates can be used to treat malignancies, and that CD30L can stimulate proliferation of T cells. Applicants

respectfully disagree that the combination of references set forth by the Examiner teaches or even suggests their invention.

Applicants have set forth in detail the reasons the first five references cited do not render their invention unpatentable. Goodwin et al. do not cure these deficiencies. They do not teach or suggest a combination of photodynamic therapy and CD40 binding proteins, nor do they suggest that CD40 binding proteins act as apoptosis-inducing agents. Applicants respectfully submit that there is no motivation in the art to make the combination of references set forth by the Examiner, nor an expectation of success in so doing. Accordingly, Applicants request that the rejection be withdrawn.

Claims 1, 2, 4 and 6 – 12 were rejected under 35 USC § 103(a), as allegedly being unpatentable over Curry et al., and/or Hunt et al., in view of Armitage et al. (US Patent 5,674,492) and/or Lynch et al., further in view of Armitage et al. (US Patent 6,410,711), and further in view of Hirano et al. The rejection is made in the interest of compact prosecution, given the election of breast cancer as a species for examination The teachings of Curry et al., Hunt et al., Armitage et al., Lynch et al. and Armitage et al. were as set forth in the previous rejection. According to the Examiner, Hirano et al. teach that CD40L can lead to decreased viability of breast carcinoma cells, due to increased apoptosis.

Applicants have set forth in detail the reasons the first five references cited do not render their invention unpatentable. Hirano et al. do not cure these deficiencies. They teach that CD40L induces apoptosis and necrosis in a carcinoma cell line. Moreover, they do not teach or even suggest a combination of photodynamic therapy and CD40 binding proteins. Applicants respectfully submit that there is no motivation in the art to make the combination of references set forth by the Examiner, nor an expectation of success in so doing. Accordingly, Applicants request that the rejection be withdrawn.

CONCLUSIONS

Claims 1 – 4, 6 - 19 and 23 – 27 are pending in this application. Claim 5 has been withdrawn as drawn to non-elected subject matter; Applicants reserve the right to petition for rejoinder of this claim when a generic claim has been found allowable. Claims 20 - 22 have been canceled, without prejudice. Applicants reserve the right to pursue such claims as in a divisional application, or rejoined to the present application. Claims 13 – 19 have not been rejected; accordingly, Applicants submit that these claims are in condition for allowance, and request notification to that effect. Newly added claims 23 – 27 are similar in format to the previously presented claims, but specify that the cells of the tumor do not express CD40. Inasmuch as the presently pending claims are believed to be in condition for allowance, Applicants respectfully request that a timely Notice to that effect be issued for this case.

Respectfully submitted,

Patricia Anne Perkins
Agent for Applicants
Registration No. 34,693
Direct Tel.: (206) 265-4782

Immunex Corporation
Law Department
1201 Amgen Court West
Seattle, WA 98119-3105
Fax: (206) 233-0644

EXHIBIT A

OIPE
MAR 19 2004
PATENT & TRADEMARK OFFICE
USPTO

UNITED STATES PATENT AND TRADEMARK OFFICE

COMMISSIONER FOR PATENTS
UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. 20231
www.uspto.gov

APPLICATION NUMBER	FILING DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	DRAWINGS	TOT CLAIMS	IND CLAIMS
09/842,745	04/25/2001	1645	1036	2922-A		22	5

CONFIRMATION NO. 7372

22932
IMMUNEX CORPORATION
LAW DEPARTMENT
51 UNIVERSITY STREET
SEATTLE, WA 98101

UPDATED FILING RECEIPT

OC000000006949324

Date Mailed: 10/22/2001

Receipt is acknowledged of this nonprovisional Patent Application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please write to the Office of Initial Patent Examination's Customer Service Center. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).**

Applicant(s)

William C. Fanslow III, Normandy Park, WA;
Elaine K. Thomas, Seattle, WA;

Assignment For Published Patent Application

IMMUNEX CORPORATION, Seattle, WA;

Domestic Priority data as claimed by applicant

THIS APPLN CLAIMS BENEFIT OF 60/199,545 04/25/2000

Foreign Applications

If Required, Foreign Filing License Granted 06/01/2001

Projected Publication Date: 01/31/2002

Non-Publication Request: No

Early Publication Request: No

Title

Method for treatment of tumors using photodynamic therapy

Preliminary Class

424

LICENSE FOR FOREIGN FILING UNDER
Title 35, United States Code, Section 184
Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Office of Export Administration, Department of Commerce (15 CFR 370.10 (j)); the Office of Foreign Assets Control, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

OIPE
MAR 19 2004
PATENT & TRADEMARK OFFICE 3060

EXHIBIT B

Entrez PubMed, English language reviews for humans, published before February 2000, with 'precancerous' in the Title

1: Luttges J, Kloppel G.
Precancerous conditions of pancreatic carcinoma.
J Hepatobiliary Pancreat Surg. 2000;7(6):568-74. Review.
PMID: 11180888 [PubMed - indexed for MEDLINE]

2: Sasatomi E, Tokunaga O, Miyazaki K.
Precancerous conditions of gallbladder carcinoma: overview of histopathologic characteristics and molecular genetic findings.
J Hepatobiliary Pancreat Surg. 2000;7(6):556-67. Review.
PMID: 11180887 [PubMed - indexed for MEDLINE]

3: Hasumi A, Matsui H, Sugioka A, Uyama I, Komori Y, Fujita J, Aoki H.
Precancerous conditions of biliary tract cancer in patients with pancreaticobiliary maljunction: reappraisal of nationwide survey in Japan.
J Hepatobiliary Pancreat Surg. 2000;7(6):551-5. Review.
PMID: 11180886 [PubMed - indexed for MEDLINE]

4: Shimonishi T, Sasaki M, Nakanuma Y.
Precancerous lesions of intrahepatic cholangiocarcinoma.
J Hepatobiliary Pancreat Surg. 2000;7(6):542-50. Review.
PMID: 11180885 [PubMed - indexed for MEDLINE]

5: Cubilla AL, Meijer CJ, Young RH.
Morphological features of epithelial abnormalities and precancerous lesions of the penis.
Scand J Urol Nephrol Suppl. 2000;(205):215-9. Review.
PMID: 11144900 [PubMed - indexed for MEDLINE]

6: Van Poppel H, Nilsson S, Algaba F, Bergerheim U, Dal Cin P, Fleming S, Hellsten S, Kirkali Z, Klotz L, Lindblad P, Ljungberg B, Mulders P, Roskams T, Ross RK, Walker C, Wersall P.
Precancerous lesions in the kidney.
Scand J Urol Nephrol Suppl. 2000;(205):136-65. Review.
PMID: 11144893 [PubMed - indexed for MEDLINE]

7: Suginoshita T, Kusuzaki K, Nagaoka T, Murata H, Hirata M, Hashiguchi S, Hirasawa Y.
Case report: natural development of osteosarcoma from precancerous lesion.
Anticancer Res. 2000 Jan-Feb;20(1B):511-4. Review.
PMID: 10769715 [PubMed - indexed for MEDLINE]

8: Genta RM, Rugge M.
Gastric precancerous lesions: heading for an international consensus.
Gut. 1999 Jul;45 Suppl 1:I5-8. Review.
PMID: 10457028 [PubMed - indexed for MEDLINE]

9: Moss SF.
Review article: Cellular markers in the gastric precancerous process.
Aliment Pharmacol Ther. 1998 Feb;12 Suppl 1:91-109. Review.
PMID: 9701007 [PubMed - indexed for MEDLINE]

10: Taddei GL, Bargelli G, Scarselli B, Moncini D, Scarselli G.
Precancerous lesions of the endometrium and medical treatment.
Eur J Contracept Reprod Health Care. 1997 Dec;2(4):239-41. Review.
PMID: 9678079 [PubMed - indexed for MEDLINE]

11: Wright JM.
A review and update of oral precancerous lesions.
Tex Dent J. 1998 Jun;115(6):15-9. Review. No abstract available.
PMID: 9667207 [PubMed - indexed for MEDLINE]

12: van der Waal I.
The diagnosis and treatment of precancerous lesions.
FDI World. 1995 Mar-Apr;4(2):6-9. Review.
PMID: 9552676 [PubMed - indexed for MEDLINE]

13: Sonnenberg A, El-Serag HB.
Economic aspects of endoscopic screening for intestinal precancerous conditions.
Gastrointest Endosc Clin N Am. 1997 Jan;7(1):165-84. Review.
PMID: 8995120 [PubMed - indexed for MEDLINE]

14: Schmitz JM, Stolte M.
Gastric polyps as precancerous lesions.
Gastrointest Endosc Clin N Am. 1997 Jan;7(1):29-46. Review.
PMID: 8995111 [PubMed - indexed for MEDLINE]

15: Li C, Zhang X, Liu W.
Recent progress in researches on precancerous lesions of gastric cancer in China.
Chin Med J (Engl). 1996 May;109(5):407-10. Review. No abstract available.
PMID: 9208502 [PubMed - indexed for MEDLINE]

16: Summerlin DJ.
Precancerous and cancerous lesions of the oral cavity.
Dermatol Clin. 1996 Apr;14(2):205-23. Review.
PMID: 8725579 [PubMed - indexed for MEDLINE]

17: Axell T, Pindborg JJ, Smith CJ, van der Waal I.
Oral white lesions with special reference to precancerous and tobacco- related lesions: conclusions of an international symposium held in Uppsala, Sweden, May 18-21 1994. International Collaborative Group on Oral White Lesions.
J Oral Pathol Med. 1996 Feb;25(2):49-54. Review.
PMID: 8667255 [PubMed - indexed for MEDLINE]

18: Koves I.
Recent management of colo-rectal tumours and precancerous conditions.
Acta Chir Hung. 1995-96;35(3-4):239-55. Review.
PMID: 9262720 [PubMed - indexed for MEDLINE]

19: Sundaresan V, Heppell-Parton A, Coleman N, Miozzo M, Sozzi G, Ball R, Cary N, Hasleton P, Fowler W, Rabbitts P.
Somatic genetic changes in lung cancer and precancerous lesions.
Ann Oncol. 1995;6 Suppl 1:27-31; discussion 31-2. Review.
PMID: 8695540 [PubMed - indexed for MEDLINE]

20: Ekbom A.
Chronic ulcerative colitis and Crohn's disease as precancerous conditions: what

is the role of colonoscopy?
Endoscopy. 1995 Jan;27(1):50-3; discussion 62-3. Review. No abstract available.
PMID: 7601036 [PubMed - indexed for MEDLINE]

21: Wright JM.
Oral precancerous lesions and conditions.
Semin Dermatol. 1994 Jun;13(2):125-31. Review.
PMID: 8060824 [PubMed - indexed for MEDLINE]

22: Lovejoy NC.
Precancerous and cancerous cervical lesions: the multicultural "male" risk factor.
Oncol Nurs Forum. 1994 Apr;21(3):497-504. Review.
PMID: 8052546 [PubMed - indexed for MEDLINE]

23: Pindborg JJ.
Clinical relevance of precancerous lesions of oral mucosa.
Recent Results Cancer Res. 1994;134:9-16. Review. No abstract available.
PMID: 8153447 [PubMed - indexed for MEDLINE]

24: Ponz de Leon M.
Genetic factors in precancerous lesions and cancer of the esophagus.
Recent Results Cancer Res. 1994;136:162-78. Review. No abstract available.
PMID: 7863094 [PubMed - indexed for MEDLINE]

25: You WC, Chang YS.
Epidemiology of precancerous gastric lesions.
J Gastroenterol Hepatol. 1993 Jul-Aug;8(4):375-82. Review.
PMID: 8374095 [PubMed - indexed for MEDLINE]

26: Reed PI, Johnston BJ.
Primary prevention of gastric precancerous lesions.
Eur J Cancer Prev. 1993 Jun;2 Suppl 2:79-82. Review. No abstract available.
PMID: 8364376 [PubMed - indexed for MEDLINE]

27: Judd PA.
Diet and precancerous lesions of the stomach.
Eur J Cancer Prev. 1993 Jun;2 Suppl 2:65-71. Review. No abstract available.
PMID: 8364374 [PubMed - indexed for MEDLINE]

28: Stockbrugger RW.
Epidemiology and pathology of precancerous lesions of the stomach.
Eur J Cancer Prev. 1993 Jun;2 Suppl 2:59-63. Review. No abstract available.
PMID: 8364373 [PubMed - indexed for MEDLINE]

29: Giacosa A, Filiberti R, Visconti P, Puntoni R.
Mediterranean diet and digestive precancerous lesions.
Eur J Cancer Prev. 1993 Jun;2 Suppl 2:17-26. Review. No abstract available.
PMID: 8364367 [PubMed - indexed for MEDLINE]

30: Nakanuma Y, Terada T, Ueda K, Terasaki S, Nonomura A, Matsui O.
Adenomatous hyperplasia of the liver as a precancerous lesion.
Liver. 1993 Feb;13(1):1-9. Review.
PMID: 8384281 [PubMed - indexed for MEDLINE]

31: Callea F, Sergi C, Fabbretti G, Brisigotti M, Cozzutto C, Medicina D.
Precancerous lesions of the biliary tree.
J Surg Oncol Suppl. 1993;3:131-3. Review.
PMID: 8389160 [PubMed - indexed for MEDLINE]

32: Hill MJ.
Diet and precancerous lesions.
Adv Exp Med Biol. 1993;348:69-74. Review. No abstract available.
PMID: 8172023 [PubMed - indexed for MEDLINE]

33: Silverman S Jr.
Precancerous lesions and oral cancer in the elderly.
Clin Geriatr Med. 1992 Aug;8(3):529-41. Review.
PMID: 1504943 [PubMed - indexed for MEDLINE]

34: Abbey LM.
Precancerous lesions of the mouth.
Curr Opin Dent. 1991 Dec;1(6):773-6. Review.
PMID: 1807482 [PubMed - indexed for MEDLINE]

35: Stich HF, Mathew B, Sankaranarayanan R, Nair MK.
Remission of precancerous lesions in the oral cavity of tobacco chewers and maintenance of the protective effect of beta-carotene or vitamin A.
Am J Clin Nutr. 1991 Jan;53(1 Suppl):298S-304S. Review.
PMID: 1985402 [PubMed - indexed for MEDLINE]

36: Mufti SI, Zirvi KA, Garewal HS.
Precancerous lesions and biologic markers in esophageal cancer.
Cancer Detect Prev. 1991;15(4):291-301. Review.
PMID: 1794136 [PubMed - indexed for MEDLINE]

37: Walt H, Emmerich P, Jauch A, DeLozier-Blanchet CD.
Characterization of precancerous and neoplastic human testicular germ cells.
Recent Results Cancer Res. 1991;123:37-44. Review. No abstract available.
PMID: 1660621 [PubMed - indexed for MEDLINE]

38: Ambros RA, Kurman RJ.
Current concepts in the relationship of human papillomavirus infection to the pathogenesis and classification of precancerous squamous lesions of the uterine cervix.
Semin Diagn Pathol. 1990 Aug;7(3):158-72. Review.
PMID: 2171125 [PubMed - indexed for MEDLINE]

39: Nicol NH.
Actinic keratosis: preventable and treatable like other precancerous and cancerous skin lesions.
Plast Surg Nurs. 1989 Summer;9(2):49-55. Review.
PMID: 2675146 [PubMed - indexed for MEDLINE]

40: Rosin MP, Dunn BP, Stich HF.
Use of intermediate endpoints in quantitating the response of precancerous lesions to chemopreventive agents.
Can J Physiol Pharmacol. 1987 Mar;65(3):483-7. Review.
PMID: 3555753 [PubMed - indexed for MEDLINE]

41: Lovejoy NC.
Precancerous lesions of the cervix. Personal risk factors.
Cancer Nurs. 1987 Feb;10(1):2-14. Review. No abstract available.
PMID: 3030539 [PubMed - indexed for MEDLINE]

42: Brescia RJ, Jenson AB, Lancaster WD, Kurman RJ.
The role of human papillomaviruses in the pathogenesis and histologic classification of precancerous lesions of the cervix.
Hum Pathol. 1986 Jun;17(6):552-9. Review.
PMID: 3011638 [PubMed - indexed for MEDLINE]

43: Koprowski H.
Embryonic precancerous and cancerous human antigens recognized by monoclonal antibodies.
Ciba Found Symp. 1983;96:204-29. Review.
PMID: 6343004 [PubMed - indexed for MEDLINE]

44: Sugimura T, Matsukura N, Sato S.
Intestinal metaplasia of the stomach as a precancerous stage.
IARC Sci Publ. 1982;(39):515-30. Review.
PMID: 6759388 [PubMed - indexed for MEDLINE]

45: Sabine JR.
Metabolic control mechanisms in precancerous liver.
Crit Rev Toxicol. 1980 Sep;7(3):189-218. Review. No abstract available.
PMID: 6250767 [PubMed - indexed for MEDLINE]

46: Koss LG.
Diagnosis of early endometrial cancer and precancerous states.
Ann Clin Lab Sci. 1979 May-Jun;9(3):189-94. Review.
PMID: 380447 [PubMed - indexed for MEDLINE]

47: Anthony PP.
Precancerous changes in the human liver.
J Toxicol Environ Health. 1979 Mar-May;5(2-3):301-13. Review.
PMID: 224200 [PubMed - indexed for MEDLINE]

48: Pindborg JJ.
Is submucous fibrosis a precancerous condition in the oral cavity?
Int Dent J. 1972 Dec;22(4):474-80. Review. No abstract available.
PMID: 4566996 [PubMed - indexed for MEDLINE]

49: Walker PR, Potter VR.
Isozyme studies on adult, regenerating, precancerous and developing liver in relation to findings in hepatomas.
Adv Enzyme Regul. 1972;10:339-64. Review. No abstract available.
PMID: 4347316 [PubMed - indexed for MEDLINE]

50: Rubin P.
Comment: Uncovering the preclinical and precancerous state.
JAMA. 1966 Feb 21;195(8):662-3. Review. No abstract available.
PMID: 5322514 [PubMed - indexed for MEDLINE]
PMID: 9834319 [PubMed - indexed for MEDLINE]

EXHIBIT C

PATENT APPLICATION SERIAL NO. _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

04/27/1999 GWASHING 00000021 60130519
01 FC:114 150.00 OP

PTO-1556
(5/87)
*U.S. GPO: 1998-433-214/80404

SERIAL NUMBER	FILING DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
60/130,519 PROVISIONAL	04/23/99		0000	273013011100

APPLICANT

MARK CURRY, VANCOUVER, CANADA.

CONTINUING DOMESTIC DATA********
VERIFIED

_____

371 (NAT'L STAGE) DATA********
VERIFIED

_____

FOREIGN APPLICATIONS********
VERIFIED

_____

IF REQUIRED, FOREIGN FILING LICENSE GRANTED 05/07/99

Foreign Priority claimed 35 USC 119 (a-d) conditions met Verified and Acknowledged	☐yes ☐no ☐yes ☐no ☐Met after Allowance Examiner's Initials _____ Initials	STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS
		CAX	1		

ADDRESS

KAWAI LAU
MORRISON AND FOERSTER LLP
2000 PENNSYLVANIA AVENUE N W
WASHINGTON DC 20006-1888

TITLE

IMMUNO-ADJUVANT PDT TREATMENT OF METASTATIC TUMORS

FILING FEE RECEIVED	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT NO. _____ for the following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit
$200		

Abstract

Immuno-adjuvant photodynamic therapy to treat and prevent metastatic cancer is effected using photosensitizers in combination with immuno-adjuvants to destroy metastatic tumor cells.

IMMUNO-ADJUVANT PDT TREATMENT OF METASTATIC TUMORS

Field of the Invention

The invention relates to the use of photodynamic therapy (PDT) treatment in combination with immuno-adjuvants to treat metastatic tumors. The PDT may be conducted with any photosensitizer, but combinations comprising a benzoporphyrin derivative (BPD) are preferred for such PDT treatment.

Description of the Related Art

This invention relates to metastatic cancer. The metastatic process, which results in the growth of secondary tumors at sites distal to the primary tumor, is the cause of death in most cancers (Poste and Fidler, 1980). Although most patients with newly diagnosed solid tumors are free of detectable metastases, and about half of those patients can be cured of their disease by local cancer treatment, the remaining patients have clinically occult micrometastases that will become evident with time. Thus, at the time of primary tumor treatment, the total percentage of patients with either detectable metastases or microscopic disseminated disease is 60% (Liotta and Stetler-Stevenson, 1989).

The brain is the most favored site for metastatic spread, occurring in 25% to 30% of all cancer patients: the most frequent primary cancers, lung cancer, breast cancer and melanoma, are associated with high incidence of brain metastases (Wright and Delaney, 1989). The lung is the second most common site of metastatic spread and pulmonary metastases most frequently originate from bone and soft-tissue sarcomas (Roth, 1989). Liver metastases commonly result from gastrointestinal tract tumors (Sugarbaker and Kemeny, 1989) and bone metastases from breast, lung and kidney primary tumors (Malawer and Delaney, 1989).

Management of a significant number of cancer cases, therefore, depends upon treating multiple tumors, traditionally through the use of surgery, radiation therapy, chemotherapy, or adjuvant therapies consisting of combinations of the three modalities.

Observations relating to tumor immunity have provided a focal point for the development of possible tumor therapy. Prehn and Main showed in 1957 that chemically induced tumors of mice were antigenic. There has been controversy concerning the relevance of chemically induced tumors, which are generally immunogenic, compared with spontaneously arising tumors in mice and human tumors which are not (Hewitt, 1979; Hewitt *et al.*, 1976).

The issue was addressed by Boon *et al.* who showed that mutagenized antigenic variants of non-immunogenic tumors could generate immunological protection in mice against the parent tumor; that is, the mutagenized and parent tumors shared antigens (Boon *et al.*, 1994). The results suggested that spontaneous experimental tumors and human tumors were antigenic and could be made immunogenic through the appropriate augmentation of the immune system (Boon *et al.*, 1994). Subsequent studies confirmed that the immune system could be made to recognize weakly immunogenic tumors by transforming tumor cells with genes for the expression of cytokines, co-stimulatory molecules, or MHC molecules (Gajewski *et al.*, 1995; Pardoll, 1993).

Also, *in vitro* culture of tumor-infiltrating lymphocytes from tumor-bearing mice and cancer patients with cytokines and irradiated tumor cells, and re-infusion of the activated lymphocytes can result in tumor regression (Burger *et al.*, 1996; Schultze *et al.*, 1997). Finally, tumor antigens recognized by the cells of the immune system have been identified in both animal models and human tumors (Jaffee and Pardoll, 1996). Tumor antigens recognized by T lymphocytes in human melanomas are the most fully characterized set of tumor antigens and may be non-mutated, widely distributed molecules, unique and mutated proteins, or normal proteins that are overexpressed in tumors (Robbins and Kawakami, 1996).

One result from the observations concerning tumor immunity is cancer immunotherapy. For centuries it has been observed that many types of diseases, including cancer, can be improved or even cured following attacks of erysipelas, an acute skin infection. In 1909 William Coley reported several positive results following

deliberate infection of cancer patients with bacteria in order to induce erysipelas. Although the contemporary theory explained tumor improvements or cures as the result of toxic products released during the bacterial infection, Coley's approach to cancer treatment may be regarded as the first instance of "biotherapy" (the original term) or cancer immunotherapy.

Immunotherapy of cancer, in which the immune system is modulated through the use of specific and non-specific tumor vaccines, bioactive molecules such as cytokines, or adoptive transfer of activated lymphocytes is one of the most appealing approaches to the treatment of metastatic cancers . The therapy is based on the concept that the patient's immunological tolerance of their cancer can be broken so that the cancer is recognized as foreign by the patient's immune system (Gore and Riches, 1996).

Another tumor treatment method is photodyanamic therapy (PDT). PDT is based upon dye-sensitized photooxidation of diseased tissue and was originally developed as a treatment modality for solid tumors (Dougherty *et al.*, 1975). Singlet oxygen ($^1O_2$) is generated, without radical formation, through energy transfer processes from light-activated photosensitizer molecules in the "type II mechanism", and it is widely accepted that $^1O_2$ is responsible for the primary photodynamic effect *in vivo* (Weishaupt *et al.*, 1976). Membrane damage brought about by $^1O_2$-mediated lipid peroxidation leading to loss of cell integrity is thought to be the primary mode of cell killing by PDT (Henderson and Dougherty, 1992), although metabolically regulated processes may also be involved in PDT-induced damage and cell death (Granville *et al.*, 1998; Tao *et al.*, 1996).

Photosensitizers are usually delivered intravenously and selective destruction of tumor tissue is based upon preferential uptake of the drug by neoplastic tissue and localized exposure of the tumor to the wavelength of light best suited to tissue penetration and photosensitizer activation. Necrosis of tumor tissue is a result of the direct effects of $^1O_2$ on tumor cells, and also from the anoxic conditions that develop in the tumor following disruption of tumor vasculature by PDT (Henderson *et al.*, 1985).

Following PDT, immune responses are initiated with the rapid induction of an inflammatory reaction (Henderson and Dougherty, 1992; Ochsner, 1997) involving the release of cytokines (Evans *et al.*, 1990; Gollnick *et al.*, 1997; Nseyo *et al.*, 1989), eicosanoids (Fingar *et al.*, 1991; Henderson and Donovan, 1989), and clotting factors (Fingar *et al.*, 1990; Foster *et al.*, 1991), and progresses to the activation of immune cells (Qin *et al.*, 1993; Yamamoto *et al.*, 1992; Yamamoto *et al.*, 1994) and infiltration of immune cells into PDT-treated tissue (Korbelik *et al.*, 1996). PDT has been shown to enhance both phagocytosis and tumor cytotoxicity when normal mouse peritoneal macrophages were treated *in vitro* (Yamamoto *et al.*, 1992; Yamamoto *et al.*, 1994) and similar treatments caused the secretion of tumor necrosis factor (TNF) (Evans *et al.*, 1990). In the clinical setting, treating bladder cancer with PDT resulted in detectable levels of interleukin (IL-1) and TNF-α in the urine of patients within 3 hours of treatment and IL-2 within 24 h in a profile that resembled treatment of bladder cancer with *Bacille Calmette Guérin* (BCG). In BCG therapy, elevated cytokine levels were associated with improvement (Evans *et al.*, 1990).

The role of the host immune system in PDT-mediated tumor eradication has recently been examined by Korbelik *et al.* by comparing the response to PDT of a solid tumor grown in immunocompetent or immunodeficient mice. PDT cured all normal mice; however, using the same treatment protocol with nude mice (which have a congenital absence of the thymus, resulting in reduced numbers of T cells but normal levels of B and NK cells) or scid mice (which are unable to complete V(D)J recombinations during T and B cell development and have no mature T and B cells), the initial tumor ablation following PDT was followed by regrowth of all of the tumors. Transferring splenic T cells to scid mice or reconstituting lethally irradiated scid mice with normal mouse bone marrow prior to PDT resulted in delayed regrowth or tumor cure (Korbelik *et al.*, 1996).

The same group observed a 200-fold increase in the number of tumor-associated neutrophils within minutes of sub-optimal photodynamic treatment and a drop in

neutrophil content to near control levels at 2 hours after light treatment (Krosl *et al.*, 1995). Infiltrating mast cell numbers also increased within 5 min of light treatment and the higher level of mast cells was maintained for 4 hours after PDT. The numbers of mast cells were, however, several logs lower than the numbers of neutrophils. Approximately 10% of the total number of cells in the tumor at 2 hours after PDT were characterized as monocytes that had invaded the tumor from the circulation.

Also, there was a large population (20% of total cells) of tumor-associated macrophages in untreated tumors. Resident macrophages were equally sensitive to PDT killing as malignant cells but following PDT, tumor associated macrophages were shown to be almost 5 times more cytotoxic against tumor target cells *in vitro*, compared with macrophages isolated from untreated tumors.

Another means of stimulating the host immune response is by the use of adjuvants. Any material that increases the immune response towards an antigen is referred to as an adjuvant (see Appendix A) and while they have been used for at least 70 years in the production of traditional vaccines designed to prevent infectious diseases, adjuvants are also being developed for use in cancer vaccines. Adjuvants are able to augment immune responses through several mechanisms including: 1) causing depot formation at the site of inoculation; 2) acting as delivery vehicles which may target antigens to cells of the immune system; 3) acting as immune system stimulators.

Many of the adjuvant preparations function via several of these mechanisms. The ideal adjuvant would have safe local and systemic reactions (which would preclude general toxicity, autoimmune and hypersensitivity reactions, and carcinogenicity) be chemically defined so consistent manufacture is possible, would enhance protective (or in the case of cancer vaccines, therapeutic) immunity towards weak antigens, and would be biodegradable (Audibert and Lise, 1993; Cox and Coulter, 1997; Gupta and Siber, 1995).

The prototypical adjuvant, which is also the most potent, is Freund's Complete Adjuvant (CFA) developed in 1937 by Jules Freund. CFA consists of a preparation of killed *Mycobacterium tuberculosis* dispersed in mineral oil. When emulsified with water

soluble antigens, the vaccine stimulates both humoral (antibody-mediated) and cell-mediated immunity towards the antigens. The use of this adjuvant may result in serious side effects including organ injury via granuloma formation and autoimmune disease, and its use is restricted even in experimental animals. Incomplete Freund's Adjuvant (IFA), which lacks the mycobacterial component of CFA, is less toxic but does not enhance cell-mediated immunity. Nonetheless, IFA is currently undergoing clinical trials in cancer vaccine formulations (for example NCI-T97-0110, NCI-98-C-0142, NCI-H98-0010, NCI-T96-0033).

New adjuvants, such as the Ribi Adjuvant System (RAS) have been designed to substitute highly purified bacterial components for *M. tuberculosis* in order to maintain the immune stimulatory properties of CFA without the side effects. A variation of RAS, Detox adjuvant is currently in clinical trials as a component of cancer vaccines (NCI-V98-1489, NCI-96-C-0139). Others, such as Hunter's TiterMax, which is has not been approved for clinical use but has been extensively characterized in animal systems, use completely synthetic compounds.

There have been previous attempts to combine immuno-adjuvants and PDT. Myers *et al.* injected formalin killed bacteria, *Corynebacterium parvum*, intralesionally in experimental tumors 24 hours prior to PDT in the first reported case of immuno-adjuvant PDT. The therapy improved the efficacy of hematoporphyrin derivative (Hpd)-sensitized PDT as measured by reduction in tumor volume and prolongation of survival (Myers *et al.*, 1989).

Using intralesional BCG, Cho *et al.* followed a similar protocol as Myers *et al.* to use PDT on a murine transitional cell carcinoma model (Cho *et al.*, 1992).

Korbelik's group reported results using immuno-adjuvant PDT in 1993 (Korbelik *et al.*, 1993). Initially, the group administered the immunostimulant schizophyllan (SPG), a glucan derived from *Schizophyllum communae*, in a series of intramuscular injections into the hind leg of mice bearing a squamous cell carcinoma solid tumor grown intradermally over the sacral region of the back. Photofrin-based PDT was administered

either 48 hours after the last SPG treatment or 24 hours before the first SPG injection. SPG therapy before PDT enhanced the effect of PDT on tumor cure whereas immunotherapy after PDT had no effect (Krosl and Korbelik, 1994).

Another study found that administering the macrophage activating factor vitamin D_3 binding protein macrophage activating factor (DBPMAF) intraperitoneally and peritumorally in a series starting immediately following Photofrin-sensitized PDT enhanced the PDT effect on tumor cures (Korbelik *et al.*, 1997). Later, the group examined the use of BCG and a purified and deproteinized preparation of the mycobacterium cell wall extract (MCWE) that is distributed by Bioniche Inc. (London, Ont. Can.) as Regressin, combined with PDT sensitized with Photofrin, Verteporfin, zinc(II)-phthalocyanine (ZnPC), and *meta*tetrahydroxyphenyl-chlorin (mThPC). A single injection of either MCWE or BCG directly beneath the tumor mass and immediately following PDT resulted in enhanced tumor cure rates (Korbelik and Cecic, 1998).

Nordquist et al. (U.S. Patent 5,747,475) disclose that the treatment of primary tumors in a rat model with indocyanine green (ICG) as chromophore and glycated chitosan as an immuno-adjuvant in photothermal therapy. This treatment resulted in some instances of reducing both primary and metastatic tumors as well as some instances of preventing the occurrence of metastatic tumors (see Figures 1 and 2 for effects against primary tumors; Figure 4 for effects against metastatic tumors; and Figure 5 for prevention of metastatic tumors).

Chen *et al.* combined glycated chitosan gel (GCG) prepared from crabshell chitin, with indocyanine green (ICG), injected ICG-GCG intratumorally and activated the ICG with thermal laser illumination in a rat metastatic tumor model. The treatment resulted in: a) no tumor response followed by death at 30 days post-treatment; b) reduced tumor burden and extended survival times to 45 days; and c) reduced tumor burden but continued growth of the treated tumor, followed by reduction of both the treated primary and untreated metastasis. Some of the animals in the last group were cured of their

tumors and rejected a subsequent challenge with the same tumor cells, indicating that the animals had developed tumor immunity and immunological memory (Chen *et al.*, 1997).

In the above instances, the processes were directed toward discrete or defined, localized tumors. Also, both Nordquist et al. and Chen et al. utilized photothermal mediated cell destruction as opposed to the photochemical mediated PDT discussed below, which does not cause any appreciable heating of the target tissue. Thus experimental combinations of immuno-adjuvants and PDT were attempted with little predictability as to actual efficacy and general application. Even the patent by Nordquist et al. only discloses the results from limited application of this concept with a single combination of one immuno-adjuvant (glycated chitosan) and one chromophore (ICG).

Given that the immune system plays an essential role in tumor destruction and the cytotoxic action of PDT, the present invention relates to a new therapeutic regime combining immunotherapy and PDT for the treatment and prevention of metastatic cancer.

Summary of the Invention

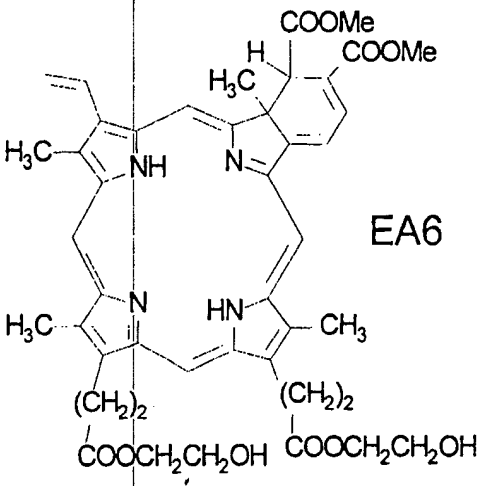
The invention is directed to the use of photodynamic therapy (PDT) in combination with immuno-adjuvants to treat, prevent, or inhibit the development of, metastatic tumors. In particular, photodynamic methods employing a photosensitizer, such as a benzoporphyrin derivative (BPD), a green porphyrin, PHOTOFRIN™ porfimer, or other hematoporphyrins, are used in combination with an immuno-adjuvant against metastatic cancer after diagnosis. Additional applications of the combination are after any primary treatment method against a diagnosed tumor to prevent the onset of as yet undetected dissemination of metastatic tumors or to treat such tumors after their appearance. The instant methods offer the benefit of efficacy against non-localized metastatic tumors either before or after their detection.

Accordingly, in one aspect, the invention is directed to a method to treat metastatic tumors, which method comprises administering to a subject with such tumors

an effective amount of a photosensitizer, such as a BPD, in combination with an immuno-adjuvant and irradiating the subject with light absorbed by the photosensitizer. Such methods may be employed against metastatic tumors upon initial diagnosis of cancer in a subject or against metastatic tumors that arise after previous tumor or cancer therapy in the subject.

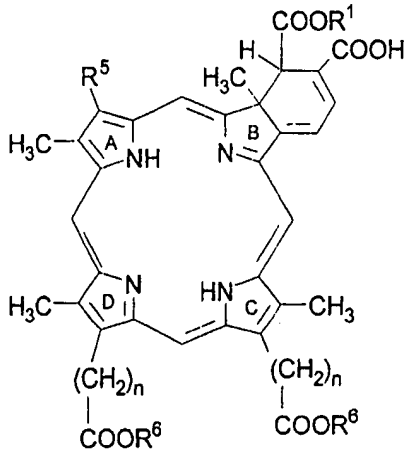
In another aspect, the invention is directed to a method to prevent or inhibit the development of metastatic tumors by the steps of administering to a subject previously having undergone cancer or tumor therapy, an effective amount of a photosensitizer, such as a BPD, in combination with an immuno-adjuvant and irradiating the subject with light absorbed by the photosensitizer. Such methods are employed even before the detection of metastasis and as such prevent, or reduce the occurrence of, metastatic tumors.

The methods of the present invention specifically are contemplated for the administration of BPDs, such as those selected from the group consisting of BPD-DA, BPD-DB, BPD-MA and BPD-MB (where BPDs are as presented in U.S. Patent 5,171,749, which is hereby incorporated by reference as if fully set forth) as well as the derivatives of these compounds. Particularly preferred BPDs include BPD-MA, EA6 and B3, where EA6 (as set forth in related application 08/852,494, which is hereby incorporated by reference as if fully set forth) and B3 (as set forth in related application 09/265,245, which is hereby incorporated by reference as if fully set forth) have the following structures.



EA6

B3 wherein R^5 is vinyl and R^1 and R^6 are methyl.



The methods of the present invention may be practiced with any immuno-adjuvant, including those set forth in Appendix A. Particularly preferred immuno-adjuvants include the Ribi Adjuvant System and TiterMax. Delivery of the immuno-adjuvant may be systemic or localized.

Regarding compositions, the present invention includes pharmaceutical compositions to treat or prevent or inhibit the development of metastatic tumors, such compositions containing an amount of a photosensitizer in combination with an immuno-adjuvant effective to treat, prevent or inhibit development of metastatic tumors when administered to a subject followed by irradiation with light absorbed by the photosensitizer, and a pharmaceutically acceptable carrier or excipient. Compositions individually containing the photosensitizer and immuno-adjuvant for use together as needed are also encompassed.

Brief Description of the Drawings

The present invention will be more clearly understood by referring to the following drawings, in which:

Fig. 1 shows biopsies containing experimental metastases in lungs of animals treated with immuno-adjuvant PDT, PDT only, and untreated controls.

Detailed Description of the Invention

The present invention is directed to a procedure in which immuno-adjuvant photodynamic therapy (PDT) targets metastatic tumors, in some instances even before they are detectable. For treating metastatic tumors that have been newly diagnosed, this treatment may be utilized as a primary therapy against the tumors. For preventing or inhibiting the development of metastatic tumors, this treatment may be used as additional or follow-up therapy after primary therapy against a diagnosed tumor.

Thus following identification of metastatic tumors in a subject, an appropriate photosensitizing compound, preferably BPD-MA, EA6 or B3, will be administered to the subject in combination with an immuno-adjuvant. The order of administration of photosensitizer and immuno-adjuvant may vary, with light irradiation following administration of the photosensitizer. The immuno-adjuvant may be administered immediately after light irradiation. Simultaneous activation of the immune system by the

immuno-adjuvant and PDT mediated damage to tumor cells, or initiation of immune reactions, may increase the effectiveness of treatment.

After administration, the photosensitizer will localize in tumor cells for photoactivation while the immuno-adjuvant proceeds to activate/potentiate the immune response. Light of appropriate frequency and intensity will be applied using an appropriate light source, thereby activating the photosensitizer to destroy tumor cells and initiate immune responses, possibly by the rapid induction of an inflammatory reaction.

The formulations and methods of the claimed invention generally relate to administering a photosensitizer, such as a green porphyrin, to a subject undergoing the immuno-adjuvant PDT. Green porphyrins are in the class of compounds called benzoporphyrin derivatives (BPD). A BPD is a synthetic chlorin-like porphyrin with various structural analogues, as shown in U.S. Patent 5,171,749. Preferably, the BPD is a benzoporphyrin derivative di-acid or mono-acid ring A (BPD-DA or BPD-MA), which absorbs light at about 692 nm wavelength with improved tissue penetration properties.

BPD-MA, for example, is lipophilic, a potent photosensitizer, and it also appears to be phototoxic to neovascular tissues, tumors and remnant lens epithelial cells. Because of its pharmokinetics, BPD-MA may be the best candidate for use in the instant invention, but other BPDs such as EA6 and B3 or other derivatives may be used instead. Other photosensitizers, such as phthalocyanines, could be used in high concentrations sufficient to offset their relatively slower uptake. An optimal BPD for immuno-adjuvant PDT treatment or prevention of metastatic tumors should be rapidly taken up by tumor cells and should be capable of initiating an immune response upon irradiation with light to act in concert with the immuno-adjuvant.

A particularly preferred formulation according to the present invention will satisfy the following general criteria. First, an immuno-adjuvant capable of activating or potentiating the immune response is utilized. Second, a photosensitizer capable of rapid entry into the target tumor cells is used. Third, irradiation with light results in

cytotoxicity to target tumor cells. This then results in the generation of immune responses. These criteria do not necessarily reflect a temporal sequence of events.

In one embodiment, the methods of the invention are used against metastatic tumors after initial diagnosis. In another embodiment, the methods of the invention follow removal or irradication of a solid tumor by conventional treatments such as surgery, radiation, chemotherapy or PDT, including immuno-adjuvant PDT. The latter embodiment may be used to prevent or inhibit the development of, metastatic tumors.

In practice of the invention, the immuno-adjuvant may be administered systemically or locally. Moreover, the immuno-adjuvant may be administered before, after or simultaneous with the photosensitizing BPD. This permits the adjuvant-mediated activation/potentiation of immune responses to overlap with PDT mediated damage to tumor cells and any PDT induced immune responses.

After administration of the photosensitizer, sufficient time is permitted to elapse for the compound to be taken up by the tumor cells. This time for uptake may be varied according to various parameters, including but not limited to the photosensitizer administered, the route of administration, the physiology of the subject and of the tumor cells, and the artisan's skill and experience. With green porphyrins, for example, the elapsed time may be from less than about one minute to more than three hours, preferably from one minute to three hours, and more preferably from 10 to 60 minutes. The cells, or tissue containing them, then are irradiated at the wavelength of maximum absorbence of the photosensitizer. In the case of BPDs, the wavelength is usually between about 550 and 695 nm, as discussed above. In particular, red light is advantageous because of its relatively lower energy and the resulting lack of toxicity it poses to normal tissue while the tumor cells are destroyed.

The compositions and methods of the present invention provide a useful immuno-adjuvant PDT treatment to treat, prevent or inhibit the development of metastatic tumors. The following describes the compositions and formulations of the present invention and their clinical application. Experimental data also are presented and described.

The Photosensitizers

The BPDs and green porphyrins useful in the method of the invention are described in detail in Levy et al., U.S. Patent No. 5,171,749 issued 15 December 1992, which is incorporated herein by reference. "Green porphyrins" refer to porphyrin derivatives obtained by reacting a porphyrin nucleus with an alkyne in a Diels-Alder type reaction to obtain a monohydrobenzoporphyrin. Typically, green porphyrins are selected from a group of porphyrin derivatives obtained by Diels-Alder reactions of acetylene derivatives with protoporphyrin under conditions that promote reaction at only one of the two available conjugated, nonaromatic diene structures present in the protoporphyrin-IX ring system (rings A and B).

Several structures of typical green porphyrins are shown in the above cited patent, which also provides details for the production of the compounds.

Dimeric forms of the green porphyrin and dimeric or multimeric forms of green porphyrin/porphyrin combinations can be used. The dimers and oligomeric compounds of the invention can be prepared using reactions analogous to those for dimerization and oligomerization of porphyrins *per se*. The green porphyrins or green porphyrin/porphyrin linkages can be made directly, or porphyrins may be coupled, followed by a Diels-Alder reaction of either or both terminal porphyrins to convert them to the corresponding green porphyrins.

Additionally, the green porphyrin compounds used in the invention may be conjugated to various ligands to facilitate targeting to target tumor cells. These ligands include those that are receptor-specific, or immunoglobulins as well as fragments thereof. Preferred ligands include antibodies in general and monoclonal antibodies, as well as immunologically reactive fragments of both.

The green porphyrin compounds of the invention may be administered as a single compound, preferably BPD-MA, or as a mixture of various green porphyrins. Suitable formulations include those appropriate for administration of therapeutic compounds *in*

vivo. Additionally, other components may be incorporated into such formulations. These include, for example, visible dyes or various enzymes to facilitate the access of a photosensitizing compound to target tumor cells.

Formulations

The photosensitizers and immuno-adjuvants of the invention may be formulated into a variety of compositions. These compositions may also comprise further components, such as conventional delivery vehicles and excipients including isotonising agents, pH regulators, solvents, solubilizers, dyes, gelling agents and thickeners and buffers and combinations thereof. Appropriate formulations and dosages for the administration of immuno-adjuvants are known in the art. Suitable excipients for use with photosensitizers and immuno-adjuvants include water, saline, dextrose, glycerol and the like.

Typically, the photosensitizing agent is formulated by mixing it, at an appropriate temperature, e.g., at ambient temperatures, and at appropriate pHs, and the desired degree of purity, with one or more physiologically acceptable carriers, *i.e.*, carriers that are nontoxic at the dosages and concentrations employed. Generally, the pH of the formulation depends mainly on the particular use, and concentration of photosensitizer, but preferably ranges anywhere from about 3 to about 8. Preferably, the photosensitizer is maintained at a pH in the physiological range (*e.g.*, about 6.5 to about 7.5). The presence of salts is not necessary, and, therefore the formulation preferably is not an electrolyte solution. Appropriate nonantigenic ingredients, such as human serum albumin, may optionally be added in amounts that do not interfere with the photosensitizing agent being taken up by lens epithelial cells.

The particular concentration of a given BPD should be adjusted according to its photosensitizing potency. For example, BPD-DA can be used but at about a five-fold higher concentration than that of BPD-MA. Moreover, the BPD may be solubilized in a different manner than by formulation in liposomes. For example, stocks of BPD-MA or

any other BPD may be diluted in DMSO (dimethylsulfoxide), polyethylene glycol or any other solvent acceptable for use in the treatment of tumors.

Normally, the adjustment of pH is not required when liposomal BPD-MA is used, as both components have a neutral pH. However, when other solvents than liposomes are used, the pH may require adjustment before mixing the BPD with the other material. Since antioxidants may interfere with the treatment, they should generally should be avoided.

Preparation of dry formulations that are reconstituted immediately before use also are contemplated. The preparation of dry or lyophilized formulations of the compositions of the present invention can also be effected in a known manner, conveniently from the solutions of the invention. The dry formulations of this invention are also storable. By conventional techniques, a solution can be evaporated to dryness under mild conditions, especially after the addition of solvents for azeotropic removal of water, typically a mixture of toluene and ethanol. The residue is thereafter conveniently dried, *e.g.* for some hours in a drying oven.

Suitable isotonising agents are preferably nonionic isotonising agents such as urea, glycerol, sorbitol, mannitol, aminoethanol or propylene glycol as well as ionic isotonising agents such as sodium chloride. The solutions of this invention will contain the isotonising agent, if present, in an amount sufficient to bring about the formation of an approximately isotonic solution. The expression "an approximately isotonic solution" will be taken to mean in this context a solution that has an osmolarity of about 300 milliosmol (mOsm), conveniently 300 + 10 % mOsm. It should be borne in mind that all components of the solution contribute to the osmolarity. The nonionic isotonising agent, if present, is added in customary amounts, i.e., preferably in amounts of about 1 to about 3.5 percent by weight, preferably in amounts of about 1.5 to 3 percent by weight.

Solubilizers such as Cremophor types, preferably Cremophor RH 40, or Tween types or other customary solubilisers, may be added to the solutions of the invention in standard amounts.

A further preferred embodiment of the invention relates to a solution comprising a BPD compound, and a partially etherified cyclodextrin, the ether substituents of which are hydroxyethyl, hydroxypropyl or dihydroxypropyl groups, a nonionic isotonising agent, a buffer and an optional solvent. However, appropriate cyclodextrins should be of a size and conformation appropriate for use with the photosensitizing agents disclosed herein.

Summaries of pharmaceutical compositions suitable for use with the instant photosensitizers and immuno-adjuvants are known in the art and are found, for instance, in Remington's Pharmaceutical Sciences.

Administration of Photosensitizers and Immuno-Adjuvants

As noted above, the treatment of the present invention is carried out in tissues either maligned with metatstatic tumors or susceptible to their occurrence, in an afflicted subject. The photosensitizer and immuno-adjuvant containing preparations of the present invention may be administered systemically or locally and may be used alone or as components of mixtures. Preferred routes of administration are intravenous, subcutaneous, intramuscular, or intraperitoneal injections of the photosensitizers and immuno-adjuvants in conventional or convenient forms. In particular, liposomal or lipophilic formulations are most desirable, and injection of the adjuvant into a tumor, whether primary or resulting from metastasis, is preferred. Intravenous delivery of photosensitizers is preferred, and intratumor injection may also be used when desired, as in pigmented tumor situations where the dose of PDT would be increased, for example. Oral administration of suitable oral formulations may also be appropriate in those instances where the photosensitizer may be readily administered to the tumor or tumor-prone tissue via this route.

Additionally, if the treatment is to be localized to an area of metastatic tumors suitable for topical formulations, the photosensitizers may be topically administered using standard topical compositions including lotions, suspensions or pastes.

The dose of photosensitizers and immuno-adjuvants can be optimized by the skilled artisan depending on factors such as, but not limited to, the physical delivery system in which it is carried, the individual subject, and the judgment of the skilled practitioner. It should be noted that the various parameters used for effective PDT in the invention are interrelated. Therefore, the dose should also be adjusted with respect to other parameters, for example, fluence, irradiance, duration of the light used in PDT, and time interval between administration of the dose and the therapeutic irradiation. All of these parameters should be adjusted to produce significant damage to metastatic tumor cells and initiate an immune response without causing significant damage to the surrounding tissue. With photosensitizers, for example, the form of administration, such as in liposomes or when coupled to a target-specific ligand, such as an antibody or an immunologically active fragment thereof, is one factor considered by a skilled artisan.

Depending on the specificity of the preparation, smaller or larger doses of photosensitizers may be needed. For compositions which are highly specific to the target tumors, such as those with the photosensitizer conjugated to a highly specific monoclonal antibody preparation or specific receptor ligand, dosages in the range of 0.05-1 mg/kg are suggested. For compositions which are less specific to the target, larger dosages, up to 1-10 mg/kg, may be desirable. The foregoing ranges are merely suggestive in that the number of variables with regard to an individual treatment regime is large and considerable deviation from these values may be expected. The skilled artisan is free to vary the foregoing concentrations so that the uptake and cellular destruction parameters are consistent with the therapeutic objectives disclosed above.

The time of immuno-adjuvant delivery may be before or after irradiation with light as well as before or after administration of the photosensitizer, although irradiation will occur after administration of the photosensitizer. The immuno-adjuvant may be delivered immediately after irradiation. This may be of particular relevance with immuno-adjuvants that are opaque or otherwise interfere with irradiation.

Without being bound by theory and in instances of BPDs being used as the photosensitizer, irradiation is thought to result in the interaction of BPD in its triplet state with oxygen and other compounds to form reactive intermediates, such as singlet oxygen, which can cause disruption of cellular structures. Possible cellular targets include the cell membrane, mitochondria, lysosomal membranes.

Each photosensitizer requires activation with an appropriate wavelength of light. With BPDs, an appropriate light source, preferably a laser or laser diode, in the range of about 550 to about 695 nm, is used to destroy target cells. An appropriate and preferred wavelength for such a laser would be 690± 12.5 nm at half maximum. Generally, cell destruction occurs within 60 seconds, and likely is sufficiently complete within about 15 to about 30 seconds. The light dose administered during the PDT treatment contemplated herein can vary, but preferably ranges between about 10 to about 150 J/cm^2. The range between about 50-100 J/cm^2 is preferred. Increasing irradiance may decrease the exposure times.

Localized delivery of light is preferred, and delivery localized to the tumor is more preferred. Delivery of light prior to photosensitizer activating light is also contemplated to improve penetration of the activating light. For example, irradiation of pigmented melanomas with infrared light before visible red light bleaches the melanin to improve penetration of the red light.

The time of light irradiation after administration of the green porphyrin may be important as one way of maximizing the selectivity of the treatment, thus minimizing damage to structures other than the target tumor cells. Light treatment immediately after application of the photosensitizer should generally be attempted.

The following examples are intended to illustrate but not to limit the invention.

Example 1

Sample Animals and Tumor Model

Male, C57BL/6 mice were obtained from Charles River Canada (Montreal, QC) at 6 to 8 weeks of age. The B16-F0 and B16-F1 melanoma cell lines were obtained from the American Type Tissue Collection (Manassas, Virginia) and grown as cell cultures in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (Sigma). The cells adhered to tissue culture plates, were removed for passage with 0.25% trypsin with 1.0 mM ethylenediaminetetraacetic acid (EDTA) (Gibco), and were cryo-preserved in liquid nitrogen in DMEM plus 40% FBS and 10% DMSO. Mice were injected with 5 X 10^5 tumor cells in a total volume of 50 µL subcutaneously into the shaved, right flank. The tumor size was monitored daily by measuring the diameter with vernier calipers and were treated when the tumors reached approximately 5 mm in diameter. In initial experiments, the B16-F0 and B16-F1 were characterized with respect to *in vivo* growth rates and metastatic potential and were found to be identical. Subsequently the B16-F1 cell line was used for all experiments.

Example 2

Sample Immuno-Adjuvant PDT

PDT treatment of mice bearing the B16-F1 tumor was performed as previously described for the M1 rhabdomyosarcoma mouse tumor (Richter *et al.*, 1987; Richter *et al.*, 1988; Richter *et al.*, 1991). Each mouse was weighed, warmed under infrared light for less than 5 min to dilate the blood vessels, restrained, and injected intravenously (tail vein) with Verteporfin at a concentration of 1.0 mg/kg body weight using a 28G needle. Thirty minutes later, animals were restrained and half of the animals were injected intratumorally with 50 µL of Titermax adjuvant (Sigma) prepared as an emulsion with sterile phosphate buffered saline (PBS) according to the manufacturers specifications. Animals were then exposed to a light dose of 100 J/cm^2 in a circular area of 1 cm diameter at 688 nm wavelength. The power density was 70 mW/cm^2 and resulted in

treatment times of 24 min per animal. Following treatment, animals were monitored daily for tumor response.

Example 3

Sample Experimental Metastases

Pulmonary metastases were generated by intravenous injection of tumor cells according to standard methods described by several groups (Chapoval *et al.*, 1998; Lin *et al.*, 1998; Volpert *et al.*, 1998; Wang *et al.*, 1998). Pulmonary metastases were initiated in each group of treated mice when the tumor was considered cured. This involved multiple treatments in some of the mice and all test animals were injected intravenously with tumor cells on the same day. Following PDT or immuno-adjuvant PDT animals were monitored for tumor response and if positive, Test (PDT and immuno-adjuvant PDT) and Control (naive) animals were injected with 5 X 10^5 tumor cells in 250 µl PBS via the lateral tail vein. The animals were monitored for tumor recurrence and general health for 14 days after which the animals were sacrificed using CO_2 inhalation and their lungs removed. Pulmonary metastases were clearly visible as black tumor colonies against the normal, pink lung tissue.

Results from the above are shown in Figure 1. The B16 melanoma tumor model is inherently difficult to treat with PDT because of the absorption of light by the black melanin pigment secreted by the tumor cells. However, 10 animals completed the entire course of the experimental procedure. Five animals received PDT alone and of those animals, 3 required repeated PDT treatments to complete the tumor cure. Five animals received immuno-adjuvant PDT and 2 required second treatments with immuno-adjuvant PDT. All of the animals that had been treated with immuno-adjuvant PDT developed between 1 and 7 lung tumors at the time of dissection. One of the animals treated with PDT alone developed 6 lung colonies but the remaining 4 animals developed between 30 and 60 lung colonies. All of the control animals developed 200 to 300 lung colonies but the density of tumor growth made accurate quantification impossible (Fig. 1).

Thus immuno-adjuvant PDT evidently augments tumor immunity that develops during tumor growth and/or following PDT. Although the above example uses pigmented tumors in an experimental metastases approach, the results indicate that the combination of an immuno-adjuvant with PDT can be used for the treatment of metastatic cancer.

All references cited hereinabove and below are hereby incorporated by reference in their entireties, whether previously specifically incorporated or not.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

References

Audibert, F.M. and Lise, L.D. (1993) Adjuvants: current status, clinical perspectives and future prospects. *Immunol Today* , 14 , 281-4.

Boon, T., Cerottini, J.C., Van den Eynde, B., van der Bruggen, P. and Van Pel, A. (1994) Tumor antigens recognized by T lymphocytes. *Annual Review of Immunology* , 12 , 337-365.

Burger, U.L., Chang, M.P., Nagoshi, M., Goedegebuure, P.S. and Eberlein, T.J. (1996) Improved in vivo efficacy of tumor-infiltrating lymphocytes after restimulation with irradiated tumor cells in vitro. *Annals of Surgical Oncology* , 3 , 580-587.

Chapoval, A.I., Fuller, J.A., Kremlev, S.G., Kamdar, S.J. and Evans, R. (1998) Combination chemotherapy and IL-15 administration induce permanent tumor regression in a mouse lung tumor model: NK and T cell-mediated effects antagonized by B cells. *J Immunol* , 161 , 6977-84.

Chen, W.R., Adams, R.L., Carubelli, R. and Nordquist, R.E. (1997) Laser-photosensitizer assisted immunotherapy: a novel modality for cancer treatment. *Cancer Letters* , 115 , 25-30.

Cho, Y.H., Straight, R.C. and Smith, J.A., Jr. (1992) Effects of photodynamic therapy in combination with intravesical drugs in a murine bladder tumor model. *J Urol* , 147 , 743-6.

Cox, J.C. and Coulter, A.R. (1997) Adjuvants--a classification and review of their modes of action. *Vaccine* , 15 , 248-56.

Dougherty, T.J., Grindley, G.B., Fiel, R., Weishaupt, K.R. and Boyle, D.G. (1975) Photoradiation therapy. II. Cure of animal tumors with hematoporphyrin and light. *Journal of the National Cancer Institute* , 55 , 115-119.

Evans, S., Matthews, W., Perry, R., Fraker, D., Norton, J. and Pass, H.I. (1990) Effect of photodynamic therapy on tumor necrosis factor production by murine macrophages. *Journal of the National Cancer Institute* , 82 , 34-39.

Fingar, V.H., Wieman, T.J. and Doak, K.W. (1990) Role of thromboxane and prostacyclin release on photodynamic therapy-induced tumor destruction. *Cancer Research* , 50 , 2599-2603.

Fingar, V.H., Wieman, T.J. and Doak, K.W. (1991) Mechanistic studies of PDT-induced vascular damage: evidence that eicosanoids mediate this process. *International Journal of Radiation Biology* , 60 , 303-309.

Foster, T.H., Primavera, M.C., Marder, V.J., Hilf, R. and Sporn, L.A. (1991) Photosensitized release of von Willebrand factor from cultured human endothelial cells. *Cancer Research* , 51 , 3261-3266.

Gajewski, T.F., Renauld, J.C., Van, P.A. and Boon, T. (1995) Costimulation with B7-1, IL-6, and IL-12 is sufficient for primary generation of murine antitumor cytolytic T lymphocytes in vitro. *Journal of Immunology* , 154 , 5637-5648.

Gollnick, S.O., Liu, X., Owczarczak, B., Musser, D.A. and Henderson, B.W. (1997) Altered expression of interleukin 6 and interleukin 10 as a result of photodynamic therapy in vivo. *Cancer Res* , 57 , 3904-9.

Gore, M. and Riches, P. (1996) The history of immunotherapy. In Gore, M. and Riches, P. (eds.), *Immunotherapy in cancer* . John Wiley & Sons, Chichester, pp. 1-9.

Granville, D.J., Levy, J.G. and Hunt, D.W. (1998) Photodynamic treatment with benzoporphyrin derivative monoacid ring A produces protein tyrosine phosphorylation events and DNA fragmentation in murine P815 cells. *Photochem Photobiol* , 67 , 358-62.

Gupta, R.K. and Siber, G.R. (1995) Adjuvants for human vaccines--current status, problems and future prospects. *Vaccine* , 13 , 1263-76.

Henderson, B.W. and Donovan, J.M. (1989) Release of prostaglandin E2 from cells by photodynamic treatment in vitro. *Cancer Research* , 49 , 6896-6900.

Henderson, B.W. and Dougherty, T.J. (1992) How does photodynamic therapy work? *Photochemistry and Photobiology* , 55 , 145-157.

Henderson, B.W., Waldow, S.W., Mang, T.S., Potter, W.R., Malone, P.B. and Dougherty, T.J. (1985) Tumor destruction and kinetics of tumor cell death in two experimental mouse tumors following photodynamic therapy. *Cancer Research* , 45 , 572-576.

Hewitt, H. (1979) A critical examination of the foundations of immunotherapy for cancer. *Clinical Radiology* , 30 , 361-369.

Hewitt, H.B., Blake, E.R. and Walder, A.S. (1976) A critique of the evidence for active host defence against cancer, based on personal studies of 27 murine tumours of spontaneous origin. *British Journal of Cancer* , 33 , 241-259.

Jaffee, E.M. and Pardoll, D.M. (1996) Murine tumor antigens: is it worth the search? *Current Opinion in Immunology* , 8 , 622-627.

Korbelik, M. and Cecic, I. (1998) Enhancement of tumour response to photodynamic therapy by adjuvant mycobacterium cell-wall treatment. *J Photochem Photobiol B* , 44 , 151-8.

Korbelik, M., Krosl, G. and Chaplin, D.J. (1993) Can PDT be potentiated by immunotherapy. *Proc. SPIE* , 1616 , 192-198.

Korbelik, M., Krosl, G., Krosl, J. and Dougherty, G.J. (1996) The role of host lymphoid populations in the response of mouse EMT6 tumor to photodynamic therapy. *Cancer Research* , 56 , 5647-5652.

Korbelik, M., Naraparaju, V.R. and Yamamoto, N. (1997) Macrophage-directed immunotherapy as adjuvant to photodynamic therapy of cancer. *British Journal of Cancer* , 75 , 202-7.

Krosl, G. and Korbelik, M. (1994) Potentiation of photodynamic therapy by immunotherapy: the effect of schizophyllan (SPG). *Cancer Letters* , 84 , 43-49.

Krosl, G., Korbelik, M. and Dougherty, G.J. (1995) Induction of immune cell infiltration into murine SCCVII tumour by Photofrin-based photodynamic therapy. *British Journal of Cancer* , 71 , 549-555.

Lin, P., Buxton, J.A., Acheson, A., Radziejewski, C., Maisonpierre, P.C., Yancopoulos, G.D., Channon, K.M., Hale, L.P., Dewhirst, M.W., George, S.E. and Peters, K.G. (1998) Antiangiogenic gene therapy targeting the endothelium-specific receptor tyrosine kinase Tie2. *Proc Natl Acad Sci U S A* , 95 , 8829-34.

Liotta, L.A. and Stetler-Stevenson, W.G. (1989) Principles of molecular cell biology of cancer: Cancer metastasis. In DeVita Jr., V.T., Hellman, S. and Rosenberg, S.A. (eds.), *Cancer: Principles and Practice* . J.B. Lippincott Company, Philadelphia, Vol. 1, pp. 98-115.

Malawer, M.M. and Delaney, T.F. (1989) Treatment of metastatic cancer Section 3: Treatment of metastatic cancer to bone. In DeVita Jr., V.T., Hellman, S. and Rosenberg, S.A. (eds.), *Cancer: Principles and Practice* . J.B. Lippincott Company, Philadelphia, Vol. 2, pp. 2298-2317.

Myers, R.C., Lau, B.H., Kunihira, D.Y., Torrey, R.R., Woolley, J.L. and Tosk, J. (1989) Modulation of hematoporphyrin derivative-sensitized phototherapy with corynebacterium parvum in murine transitional cell carcinoma. *Urology* , 33 , 230-235.

Nseyo, U.O., Whalen, R.K., Duncan, M.R., Berman, B. and Lundahl, S. (1989) Immune response following photodynamic therapy for bladder cancer. *Proceedings of the Society of Photo-Optical Instrumentation Engineers* , 1065 , 66-72.

Ochsner, M. (1997) Photophysical and photobiological processes in the photodynamic therapy of tumours. *Journal of Photochemistry and Photobiology. B, Biology* , B - Biology. 39 , 1-18.

Pardoll, D.M. (1993) New strategies for enhancing the immunogenicity of tumors. *Current Opinion in Immunology* , 5 , 719-725.

Poste, G. and Fidler, I.J. (1980) The pathogenesis of cancer metastasis. *Nature* , 283 , 139-145.

Qin, B., Selman, S.H., Payne, K.M., Keck, R.W. and Metzger, D.W. (1993) Enhanced skin allograft survival after photodynamic therapy. Association with lymphocyte inactivation and macrophage stimulation. *Transplantation* , 56 , 1481-1486.

Richter, A.M., Kelly, B., Chow, J., Liu, D.J., Towers, G.H.N., Dolphin, D. and Levy, J.G. (1987) Preliminary studies on a more effective phototoxic agent than hematoporphyrin. *Journal of the National Cancer Institute* , 79 , 1327-1332.

Richter, A.M., Sternberg, E., Waterfield, E., Dolphin, D. and Levy, J.G. (1988) Characterization of benzoporphyrin derivative, a new photosensitizer. *Proceedings of the Society of Photo-Optical Instrumentation Engineers* , 997 , 132-138.

Richter, A.M., Yip, S., Waterfield, E., Logan, P.M., Slonecker, C.E. and Levy, J.G. (1991) Mouse skin photosensitization with benzoporphyrin derivatives and Photofrin: macroscopic and microscopic evaluation. *Photochemistry and Photobiology* , 53 , 281-286.

Robbins, P.F. and Kawakami, Y. (1996) Human tumor antigens recognized by T cell. *Current Opinion in Immunology* , 8 , 628-636.

Roth, J.A. (1989) Treatment of metastatic cancer Section 2: Treatment of metastatic cancer to lung. In DeVita Jr., V.T., Hellman, S. and Rosenberg, S.A. (eds.), *Cancer: Principles and Practice* . J.B. Lippincott Company, Philadelphia, Vol. 2, pp. 2261-2275.

Schultze, J.L., Seamon, M.J., Michalak, S., Gribben, J.G. and Nadler, L.M. (1997) Autologous tumor infiltrating T cells cytotoxic for follicular lymphoma cells can be expanded in vitro. *Blood* , 89 , 3806-3816.

Sugarbaker, P.H. and Kemeny, N. (1989) Treatment of metastatic cancer Section 3: Treatment of metastatic cancer to liver. In DeVita Jr., V.T., Hellman, S. and Rosenberg, S.A. (eds.), *Cancer: Principles and Practice* . J.B. Lippincott Company, Philadelphia, Vol. 2, pp. 2275-2298.

Tao, J., Sanghera, J.S., Pelech, S.L., Wong, G. and Levy, J.G. (1996) Stimulation of stress-activated protein kinase and p38 HOG1 kinase in murine keratinocytes following

photodynamic therapy with benzoporphyrin derivative. *Journal of Biological Chemistry* , 271 , 27107-27115.

Volpert, O.V., Lawler, J. and Bouck, N.P. (1998) A human fibrosarcoma inhibits systemic angiogenesis and the growth of experimental metastases via thrombospondin-1 . *Proc Natl Acad Sci U S A* , 95 , 6343-8.

Wang, J., Saffold, S., Cao, X., Krauss, J. and Chen, W. (1998) Eliciting T cell immunity against poorly immunogenic tumors by immunization with dendritic cell-tumor fusion vaccines. *J Immunol* , 161 , 5516-24.

Weishaupt, K., Gomer, C.J. and Dougherty, T.J. (1976) Identification of singlet oxygen as the cytotoxic agent in photo- inactivation of a murine tumor. *Cancer Research* , 36 , 2326-2329.

Wright, D.C. and Delaney, T.F. (1989) Treatment of metastatic cancer Section 1: Treatment of metastatic cancer to the brain. In DeVita Jr., V.T., Hellman, S. and Rosenberg, S.A. (eds.), *Cancer: Principles and Practice* . J.B. Lippincott Company, Philadelphia, Vol. 2, pp. 2245-2261.

Yamamoto, N., Hoober, J.K. and Yamamoto, S. (1992) Tumoricidal capacities of macrophages photodynamically activated with hematoporphyrin derivative. *Photochemistry and Photobiology* , 56 , 245-250.

Yamamoto, N., Sery, T.W., Hoober, J.K., Willett, N.P. and Lindsay, D.D. (1994) Effectiveness of photofrin II in activation of macrophages and in vitro killing of retinoblastoma cells. *Photochemistry and Photobiology* , 60 , 160-164.

Appendix A: Adjuvant Classification

PARTICULATE ADJUVANTS

-exist as microscopic, insoluble particles

-generally, the immunogen must be incorporated into or associated with the particle.

A. Mineral-based

-insoluble, gel-like precipitate

-mineral formulations are the only adjuvants that are considered safe and effective for use in human vaccines

i. **Aluminum hydroxide (Alhydrogel)**
Superfos chemicals
http://www.superfos.com/index.htm

a. **SBAS4**
Aluminum salt combined with monophosphoryl lipid A (MPL)
SmithKline Beecham
http://www.sb.com/index.html

ii. **Aluminum phosphate (Adju-Phos)**
Superfos chemicals
http://www.superfos.com/index.htm

ii. **Calcium phosphate**
Superfos chemicals
http://www.superfos.com/index.htm

B. Water-in-oil emulsions

-microdroplets of water, stabilized by surfactant in a continuous oil phase

i. **Freund's Complete Adjuvant (FCA)**
a mixture of a non-metabolizable oil (mineral oil), a surfactant (Arlacel A), and mycobacteria (*M. tuberculosis* or *M. butyricum* in Modified FCA)
Superfos chemicals
http://www.superfos.com/index.htm

ii. **Freund's Incomplete Adjuvant (FIA)**
has the same oil/surfactant mixture as FCA but does not contain any mycobacteria

iii. **Montanide Incomplete Seppic Adjuvant (ISA) Adjuvants**
a group of oil/surfactant based adjuvants in which different surfactants
are combined with either a non-metabolizable mineral oil, a metabolizable oil, or a mixture of the two. They are prepared for use as an emulsion with aqueous Ag solution. The surfactant for Montanide ISA 50 is mannide oleate, a major component of the

surfactant in Freund's adjuvants. The surfactants of the Montanide group undergo strict quality control to guard against contamination by any substances that could cause excessive inflammation, as has been found for some lots of Arlacel A used in Freund's adjuvant. The various Montanide ISA group of adjuvants are used as water-in-oil emulsions, oil-in-water emulsions, or water-in-oil-in-water emulsions. The different adjuvants accommodate different aqueous phase/oil phase ratios, because of the variety of surfactant and oil combinations. The performance of these adjuvants is said to be similar to Incomplete Freunds Adjuvant for antibody production; however the inflammatory response is usually less.
Seppic, Paris, France

C. **Oil-in-water emulsions**
-microdroplets of squalene or squalane, stabilized with surfactants in a continuous water phase, developed for human clinical trials when combined with immunomodulators

i. **Ribi Adjuvant System (RAS)**
4 components: (1) monophosphoryl lipid A (MPL); (2) trehalose dimycolate (TDM); (3) cell wall skeletons (CWS); (4) *S. typhimurium* mitogen (STM)
Ribi ImmunoChem Research, Inc.
http://www.ribi.com/

ii. **MF59**
originally developed with N-acety-muramyl-L-alanyl-2-(1',2'-dipalmitolyl-sn-glycero-3'-phospho)ethylamide (MTP-PE) however when antibody titer was endpoint, MTP-PE was not required for adjuvant activity
Chiron Corp.
http://www.chiron.com/

iii. **SBAS4**
combination of monophosphory lipid A (MPL), QS21, and a proprietary oil in water emulsion
SmithKline Beecham
http://www.sb.com/index.html

D. **Immune stimulating complexes (ISCOM)**
-open, cage-like structure resulting from the interaction of Quil-A with cholesterol and phosphatidycholine, human clinical trials

E. **Liposomes**
-single or multilamellar bilayer membrane vesicles comprised of cholesterol and phospholipid
-the immunogen may be membrane-bound or within the intermembrane spaces

F. **Nano- and microparticles**

-solid particles, biocompatible and biodegradable, synthetic polymers of cyanoacrylates, polycatide coglycolide (PLG) copolymer, antigen must be formulated with particle

NON-PARTICULATE ADJUVANTS

A. **Muramyl dipeptide (MDP) and derivatives: Adjuvant peptides**

-*N*-acetyl muramyl-L-alanyl-D-isoglutamine is the active component of peptidoglycan extracted from *Mycobacterium*, derivatives are less toxic

i. **threonyl MDP**

ii. **murabutide,*N*-acetylglucosaminyl-MDP (GMDP)**

a. Gerbu Adjuvant
Alternative to FCA. Oil is replaced by water-soluble, aliphatic quaternary amines or bio-degradable esterquats. Mycobacterium is replaced by GMDP.
Gerbu Biotechnik GmbH, Gaiberg, Germany
C-C Biotech
16766 Espola Road
Poway, CA 92064
USA

iii. **murametide**

iv. **nor-MDP**

B. **Non-ionic block copolymers**

-polymers composed of a region of hydrophobic polyoxypropylene (POP) flanked by regions of hydrophilic polyoxyethylene (POE), not biodegradable

i. **TiterMax**
CytRx Corporation
http://www.cytrx.com/

CytRx owns 87.5% of Vaxcel, Inc. (OTCBB: VXCL) which is engaged in the development and commercialization of vaccine adjuvants and delivery systems and a novel vaccine for the treatment of cancer. Vaxcel has four proprietary adjuvant and delivery system technologies which can be used to increase the effectiveness and/or convenience of both currently marketed and new vaccines. These four technologies are complementary to each other and provide Vaxcel with a broad portfolio of technologies for the development of vaccines to be delivered by the injectable, oral and mucosal routes of administration. Vaxcel's business strategy is to sublicense these adjuvant and delivery system technologies on a vaccine-by-vaccine basis to companies engaged in vaccine development. Vaxcel has retained Interstate/Johnson Lane

Corporation to introduce Vaxcel and its technologies to the trade with the purpose of concluding a strategic transaction.

iv. **Syntex Adjuvant Formulation-1 (SAF-1)**
Roche Bioscience (formerly Syntex Corp., Palo Alto, CA)
http://www.roche.com/pharma/Index.htm

iv. **SAF-2**

C. **Saponins**
-extract of Quillaia saponaria tree, saponin is crude extract of triterpenoids

i. **Quil A**
Partially purified saponin

ii. **Spikoside**
Partially purified saponin

iii. **QS21 (Stimulon)**
Purified, defined entity
Aquila Biopharmaceuticals, Inc. (formerly Cambridge Biotech Corporation)
http://www.aquilabio.com/

iv. **ISCOPREP™ 703**
Purified, defined entity

D. **Lipid A and derivatives**
-disaccharide of glucosamine with two phosphate groups and five or six fatty acid chains (C_{12} to C_{16} in length)

i. **monophosphoryl lipid A (MPL)**
removal of the 1' phosphate group from lipid A gives MPL

E. **Cytokines**

F. **Carbohydrate polymers**
-polymers of mannose and β1-3 glucose
-proposed as human vaccine adjuvants either mixed with or conjugated with immunogen
-stimulate macrophages and dendritic cells

G. **Derivatized polysaccharides**
-high molecular weight sulphated dextrans proposed as human vaccine adjuvants

H. **Bacterial toxins**
-potent mucosal adjuvants in animal models

We claim:

1. A method of treating metastatic tumors in a subject, which method comprises:

administering to a subject afflicted by metastatic tumors effective amounts of a photosensitizer and an immuno-adjuvant, and

irradiating said subject with light absorbed by said photosensitizer,

wherein said method is photochemical mediated photodynamic therapy (PDT).

2. A method of preventing or inhibiting the development of metastatic tumors in a subject, which method comprises:

administering to a subject at risk for developing metastatic tumors effective amounts of a photosensitizer and an immuno-adjuvant, and

irradiating said subject with light absorbed by the photosensitizer.

3. A method of treating a primary tumor in a subject, which method comprises:

administering to a subject clinically diagnosed with a primary tumor effective amounts of a photosensitizer and an immuno-adjuvant, and

irradiating said subject with light absorbed by said photosensitizer.

4. The method of claim 2 wherein said subject has previously undergone cancer or tumor therapy.

5. The method of claims 1, 2 or 3 wherein said effective amount of a photosensitizer is in the range of 0.05 to 10 mg/kg.

6. The method of claim 5 wherein said effective amount of a photosensitizer is in the range of 0.05 to 1 mg/kg.

7. The method of claim 5 wherein said effective amount of a photosensitizer is in the range of 1 to 10 mg/kg.

8. The method of claims 1 or 3 wherein said photosensitizer is administered intravenously and said immuno-adjuvant is administered by injection into tumors.

9. The method of claims 1 or 3 wherein said irradiation is localized to the tumors.

10. The method of claim 2 wherein said photosensitizer is administered intravenously or intratumorally.

11. The method of claims 1, 2 or 3 wherein said photosensitizer is administered, and the subject irradiated, before administration of the immuno-adjuvant.

12. The method of claims 1, 2 or 3 wherein said immuno-adjuvant is administered systemically.

13. The method of claims 1, 2 or 3 wherein the photosensitizer is a benzoporphyrin derivative (BPD), a green porphyrin or a hematoporphyrin.

14. The method of claim 13 wherein the BPD is BPD-MA, EA6, or B3.

15. The method of claims 1, 2 or 3 further comprising an additional irradiation, before irradiation with light absorbed by the photosensitizer, with light of a wavelength which improves penetration of the absorbed light.

16. A pharmaceutical composition to treat, prevent, or inhibit the development of, metastatic tumors, said composition comprising:

a photosensitizer and an immuno-adjuvant in amounts effective to treat, prevent, or inhibit the development of, metastatic tumors, and

a pharmaceutically acceptable carrier or excipient.

17. The composition of claim 16 wherein the photosensitizer is a BPD, a green porphyrin or a hematoporphyrin.

18. The composition of claim 17 which is a liposomal formulation.

19. The composition of claim 17 wherein the BPD is BPD-MA, EA6, or B3.

09103514.042303

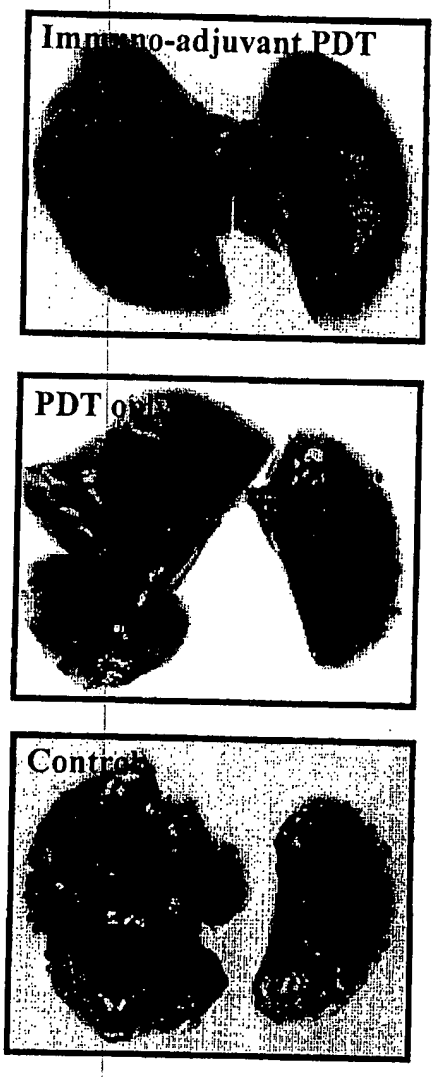


Figure 1. Experimental metastases in lungs of animals treated with immuno-adjuvant PDT, PDT, and untreated controls.

PTO/SB/16 (6-95) (modified)
Approved for use through 04/11/98. OMB 0651-0037
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 C.F.R. § 1.53(b)(2).

04/23/99 jc595 U.S. PTO

jc541 U.S. PTO 60/130519 04/23/99

CERTIFICATE OF HAND DELIVERY

I hereby certify that this correspondence is being hand filed with the United States Patent and Trademark Office in Washington, D.C. on April 23, 1999.

Karyn S. Hines

Karyn S. Hines

Docket Number	273013011100	Type a plus sign (+) inside this box	+

INVENTOR(s)/APPLICANT(s)

LAST NAME	FIRST NAME	MID INIT	RESIDENCE (CITY AND STATE/FOREIGN COUNTRY)
Curry	Mark		

TITLE OF THE INVENTION (280 characters max)

IMMUNO-ADJUVANT PDT TREATMENT OF METASTATIC TUMORS

CORRESPONDENCE ADDRESS

Kawai Lau
Morrison & Foerster LLP
2000 Pennsylvania Avenue, N.W.
Washington, D.C. 20006-1888
(202) 887-6939

ENCLOSED APPLICATION PARTS (check all that apply)

- ☒ Specification Number of Pages 35
- ☒ Drawing(s) Number of Sheets 1
- ☐ Small Entity Statement
- ☐ Other (specify) _____

METHOD OF PAYMENT (check one)

- ☒ A check or money order is enclosed to cover the Provisional filing fees.
- ☐ The Assistant Commissioner is hereby authorized to charge filing fees and credit Deposit Account No.: 03-1952.

PROVISIONAL FILING FEE AMOUNT($): $150.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

- ☒ No.
- ☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

SIGNATURE _____ Date: April 23, 1999

TYPED or PRINTED NAME: Kawai Lau

REGISTRATION NO.: P-44,461

☐ Additional inventors are being named on separately numbered sheets attached hereto.

USE ONLY FOR A PROVISIONAL APPLICATION FOR PATENT

1181 0G 62 (12/95)
dc-156761

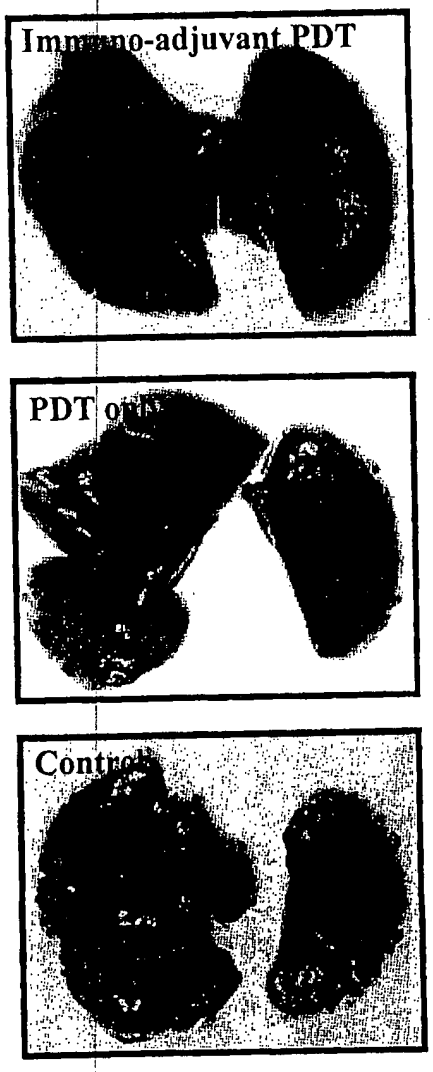


Figure 1. Experimental metastases in lungs of animals treated with immuno-adjuvant PDT, PDT, and untreated controls.